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Inactivation of *E. coli* and *E. faecalis* by solar photo-Fenton with EDDS complex at neutral pH in municipal wastewater effluents

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ABSTRACT

Photo-Fenton is a solar disinfection technology widely demonstrated to be effective to inactivate microorganisms in water by the combined effect of photoactivated iron species and the direct action of solar photons. Nevertheless, the precipitation of iron as ferric hydroxide at basic pH is the main disadvantage of this process. Thus, challenge in photo-Fenton is looking for alternatives to iron salts. Polycarboxylic acids, such as Ethylendiamine-N',N'-disuccinic acid (EDDS), can form strong complex with Fe^{3+} and enhance the dissolution of iron in natural water through photochemical process. The aim of this study was to evaluate the disinfection effectiveness of solar photo-Fenton with and without EDDS in water. Several reagent concentrations were assessed, best bacterial (*Escherichia coli* and *Enterococcus faecalis*) inactivation was obtained with 0.1:0.2:0.3 mM (Fe^{3+}:EDDS:H_2O_2) within isotonic water. The benefit of using EDDS complexes to increase the efficiency of kept dissolved iron in water at basic pH was proved. Solar disinfection and H_2O_2/solar with and without EDDS, and Fe-EDDS complexes were also investigated. Bacterial inactivation results in municipal wastewater effluents (MWWE) demonstrated that the competitive role of organic matter and inorganic compounds strongly affect the efficacy of Fe:EDDS at all concentrations tested, obtaining the faster inactivation kinetics with H_2O_2/solar (0.3 mM).

1. Introduction

Water purification is one of the greatest challenges of the 21st century due to water scarcity, reduction of ground water source by chemical and biological pollution and the increased water human's demand. To address it, the reuse of wastewater appears as a possible solution for activities like irrigation, industrial applications, domestic and environmental uses. Currently, reuse of treated wastewater is increasing in countries like Australia, Spain, Italy, USA (California and Florida), etc., following their own guidelines and regulations [1–3]. Water reuse offers a climate independent water source, locally-managed, and generally beneficial to the environment. This water strategy balances increasing population growth, dry climates and the high irrigation/agricultural demands. Additionally, water reuse may reduce the nutrient loads from wastewater discharges into waterways, therefore reducing and preventing pollution [3].

Nevertheless, the presence of pollutants (chemical and biological) in wastewater effluents make the treatment of secondary effluents necessary before being reused. In line with this, Advanced Oxidation Processes (AOPs) have become innovative and successful wastewater treatments for both, decontamination and disinfection, with the advantage that they can be driven by either artificial or solar UV energy [4]. The efficiency of these treatments lies on the generation of hydroxyl radicals ($HO^{\bullet}$). Amongst solar driven-AOPs, photo-Fenton has shown high efficiency in removing chemical pollutants and waterborne pathogens from water resources. It consists on a number of catalytic reactions involving iron salt, hydrogen peroxide (H_2O_2) and electromagnetic radiation ($\lambda > 580$ nm) generating $HO^{\bullet}$ mainly by the following

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reactions [5]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO^{\bullet}\left(\mathrm{K} = 70\mathrm{M}^{-1}\mathrm{S}^{-1}\right) \quad (1)$$

$$Fe(OH)^{2+} + hv \rightarrow Fe^{2+} + HO^{\bullet} \quad (2)$$

Regarding photo-Fenton's efficiency, pH is one of the most critical parameters since the formation of photoactive iron complexes is highly dependant on water pH. Optimal pH is equal to 2.8; at this pH no iron precipitation occurs, and dominant iron species present in water $(Fe(OH))^{2+}$ are the lightest active hydroxycomplex species [6]. Water treatment at pH 2.8 requires acidification and neutralization as pre- and post-treatment, respectively with the corresponding reagents consumption. Moreover, acid pH values are toxic for microorganisms and environment, therefore the current challenge of this solar technique is to accomplish a good disinfection performance of photo-Fenton at near neutral pH [6,7].

In this scenario, research has been focused on the use of iron complexes. It is known that polycarboxylates, including citrate, malonate and oxalate, can form strong complex with Fe^{3+} and enhance the dissolution of iron in natural water through photochemical process [8–10]. Besides polycarbxylates, aminopolycarboxylic acids (APCAs) may present a similar behavior. Amongst the APCAs, EDTA (ethilendiaminetetracetic acid) has been widely used as Fe^{3+} complexing to neutral pH conditions since EDTA forms a chelated soluble complex. However, it is considered as a contaminant due to its low biodegradability and its application has been limited. Ethylendiamine-N',N'-disuccinic acid (EDDS), has been reported as a good candidate to form Fe chelated soluble complex and it is safe to environment and biodegradable [9,10]. EDDS is a structural isomer of EDTA, it exists in three stereo isomers (S,S)-EDDS, (R,R)-EDDS and (R,S/S,R)-EDDS, being (S,S)-EDDS readily biodegradable [11]. The EDDS complex keeps Fe^{3+} in solution, thus it is active for the process. Irradiated solutions of Fe^{3+}-EDDS generate $HO^{\bullet}$ according to the following reactions [12]:

$$Fe^{3+} - EDDS + hv \rightarrow Fe^{2+} + EDDS^{\bullet} \quad (3)$$

$$Fe^{2+} \xrightarrow{EDDS,O_2} \left[Fe^{3+} - EDDS\right]^- \quad (4)$$

$$EDDS^{\bullet 3-} + OH^- \rightarrow EDDS^{4-} + HO^{\bullet} \quad (5)$$

Furthermore, other Reactive Oxygen Species (ROS), such as $O_2^{\bullet -}$ and $HO_2^{\bullet}$, are generated due to the iron and aminopolycarboxylic acid complex reactions in the presence of oxygen [8,13]:

$$EDDS^{\bullet} + O_2 \rightarrow EDDS' + O_2^{\bullet -} \quad (6)$$

$$O_2^{\bullet -} + H_3O^+ \rightarrow HO_2^{\bullet} + H_2O \quad (7)$$

The benefits of using EDDS complex in photo-Fenton for water and wastewater decontamination have been reported in literature [13–17]. It has been described that EDDS complex increases the efficiency of photo-Fenton compared with traditional iron salts due to the fact that complexation between iron and EDDS favors the solubility and reactivity of iron at neutral pH. The Fe:EDDS complex quantum yield of $HO^{\bullet}$ ($\Phi_{HO^{\bullet}}$) generation in the range of pH 3–8 was investigated by Li et al. [9]. These authors reported that the $\Phi_{HO^{\bullet}}$ increased when raising water pH from 0.0025 (pH 3) to 0.069 (pH 9) for a concentration of 10^{-4} M Fe-EDDS at $\lambda = 365$ nm [9].

Nevertheless, according to Giannakis et al. [6,7], up to date, photo-Fenton application for wastewater disinfection has not been deeply investigated. First contribution by Klamerth et al. [18] reported data regarding removal of micro-pollutants and total coliforms present in real wastewater effluents under natural sunlight using EDDS complex. Recently, it has been reported in literature that the complex Fe(III)-EDDS show a dual role on water disinfection, i.e., it benefits the generation of radical species at neutral pH but also acts as a trap for these same radicals, highlighting also that the concentration of Fe(III)-EDDS complex is a key parameter for the inactivation of microorganisms in water [19].

The aim of this study was to evaluate the effect of EDDS-complex in the efficiency of photo-Fenton treatment conducted by solar radiation in comparison with the traditional photo-Fenton at near-neutral pH. The effect of several concentrations of Fe:EDDS and H_2O_2 on the inactivation efficiency of *E. coli* and *E. faecalis* as well as the effect of pH in isotonic water have been evaluated. Boundary treatments of photo-Fenton such as solar light, H_2O_2/solar irradiation, Fe^{3+}/solar irradiation with and without the presence of EDDS have been also investigated. In addition, the efficiency of the use of EDDS complex in MWWE for water disinfection has been also investigated at several reagent´s concentration.

2. Materials and methods

2.1. Water source

Isotonic water (IW), i.e, demineralized water with 0.9% of NaCl was used as water model without the interference of other chemical compounds (organic and inorganic). IW main characteristics: pH ~ 6, conductivity < 10μS/cm, Cl^- = 0.7-0.8 mg/L, NO_3^- = 0.5 mg/L and dissolved organic carbon (DOC) < 0.5 mg/L.

Secondary effluent from the Municipal Wastewater Treatment Plant (MWWE) of "El Bobar" (Almería, Southeast of Spain) was also used. Several batches were daily collected. Its main characteristics were: pH ~7.5, conductivity 1500 μS/cm, turbidity 8–16 NTU, DOC 15–30 mg/L, *E. coli* concentration ca. 2.5×10^3 CFU/mL and *E. faecalis* 1.0×10^2 CFU/mL. DOC were measured by direct injection of filtered samples with 0.2 μm Nylon filter into TOC-VCSN (Shimadzu), turbidity was measured with a turbidimeter (Model 2100 N, Hach, USA), and pH with WTW probe (Germany, series multi 720).

2.2. Bacterial strains enumeration and quantification

Escherichia coli K-12 (Gram-negative bacteria) (ATCC 23631) and *Enterococcus faecalis* (Gram-positive bacteria) (CECT 5143) were used as bacterial models and spiked in IW experiments. Enumeration-quantification method used to attain an initial bacterial concentration of ~10^6 CFU/mL has been described previously [19,20]. The samples taken during IW experiment were enumerated using the standard plated counting method through a serial 10 fold dilutions in Phosphate Buffer Solution (PBS) and volumes of 20 μL were plated in triplicate on Luria agar supplemented with Sodium duodecil sulphate or Slanetz Bartley agar (Scharlau®, Spain) for *E. coli* or *E. faecalis*, respectively. Colonies were counted after incubation of 24–48 h at 37 °C. Detection Limit (DL) was 4 CFU/mL. In MWWE, a selective and differential agar for naturally occurring bacterial enumeration was used: Chromocult® Coliform Agar (Merck KGaA, Germany) and Slanetz Bartley agar for *E. coli* and *E. faecalis,* respectively. Plate counting technique spreading 50-250-500 μL of sample was used, reaching a DL of 2 CFU/mL. After 24 h (at 44 °C) and 48 h (at 37 °C), colonies of *E. coli* and *E. faecalis* were counted.

2.3. Reagents

Hydrogen peroxide (H_2O_2, 35% w/v, Riedel-de Häen, Germany) at concentrations of 0.15 and 0.30 mM were used according to previous work [20]. H_2O_2 concentration was followed by spectrophotometry (PG instruments Ltd T-60-U) according to DIN (38409 H15), based on the formation of a yellow complex from the reaction of titanium (IV) oxysulfate with H_2O_2 at 410 nm. Ferric sulfate ($Fe_2(SO_4)_3{\cdot}H_2O$) (Panreac, Spain) was used as iron source. Iron concentration was determined by spectrophotometry at 510 nm according to ISO 6332. Ethylendiamine-N',N'-disuccinic acid (EDDS: $C_{10}H_{16}N_2O_8$, Aldrich, USA) was used to complex iron. The concentrations tested were 0.05, 0.2 and 0.3 mM according to previous work [16,17,19]. The DOC contributions to the water by EDDS were 6, 24 and 36 mg/L, respectively. NaOH (J.T.Baker, Holland) was used for pH adjustment. Bovine catalase (Sigma) (0.1 g/L) was added to the samples (20 μL of catalase for 1 mL of sample) to remove residual H_2O_2 [19].

2.4. Solar experiments

Solar experiments were done with 250-mL DURAN-glass (Schott, Germany) vessel reactors magnetically stirred at 100 rpm [20]. UV-A transmission (borosilicate glass) was 90% (cut-off at 280 nm). Total irradiate volume was 0.2 L and illuminated surface 0.0095 m^2. All experiments were carried out at Plataforma Solar de Almería (Almería, Spain) in triplicate under completely sunny conditions. The Fe:EDDS solution was daily prepared before each solar disinfection experiment according to previous works [16–19]. It consists on dissolving $Fe_2(SO_4)_3{\cdot}H_2O$ in water at pH3, adding a determined volume of a EDDS solution and homogenizing the suspension for 15 min in dark. Concentration of EDDS solution and iron were added according to the reagent concentration required in each case. After that, the complex formed was directly diluted in the reactor to achieve the required initial concentration. Reagents and microbial suspensions were added in the dark to the solar reactor. Temperature, pH, H_2O_2 and iron were measured periodically. When H_2O_2 concentration was below 0.03 mM, additional H_2O_2 dosing (similar to the initial one) was added to avoid limitation by lack of H_2O_2. pH was 6 or 8 for photo-Fenton and Fe:EDDS assays. In IW experiments, *E. coli* and *E. faecalis* were spiked in the water and determined simultaneously. All initial samples were kept in the dark at room temperature and re-plated at the end of the experiment as control sample to guarantee strain good quality (no concentration decrease). In MWWE experiments, naturally occurring *E. coli* and *E. faecalis* were also investigated simultaneously. pH was natural (~7.5). Water samples were taken every 15 min, adding catalase and analyzing as described above. Dark tests using the same operational conditions as solar treatments were done to discard toxicity effect over pathogens viability. Regrowth counts of pathogens were determined for all the experiments by leaving the last two samples at room temperature for 24 and 48 h. Results were analyzed through one-way ANOVA ($P < 0.05$, Origin v7.03, OriginLab Corp., 30 Northampton, USA), reporting a 95% confidence level for the average colony concentration error.

2.5. Solar radiation

UV radiation was measured with a global UV-A pyranometer which provides data in terms of incident W/m^2 and was plotted as Q_{UV} (accumulated UV energy per unit of volume of treated water (kJ/L) received in the photoreactor in a given time). This factor allows normalizing the energy available for the photocatalytic reaction under natural sunlight according to Eq. (8):

$$Q_{UV,n} = Q_{UV,n-1} + \frac{\Delta t_n \overline{UV}_{G,n} A_r}{V_t}; \Delta t_n = t_n - t_{n-1}$$

Where $Q_{UV,n}$, $Q_{UV,n-1}$ is the UV energy accumulated per unit volume (kJ/L) at times *n* and *n-1,* respectively, $UV_{G,n}$ is the average incident irradiation on the irradiated area, Δt_n is the experimental time of sample, A_r is the illuminated area of the bottle reactor (m^2), and V_t is the total volume of treated water (L).

2.6. Kinetics

All experimental data from disinfection tests were fitted according to the following kinetics models: 1) A log-linear according to the Chick´s law (Eq. (9)). 2) An initial delay or very smooth decay at the beginning ('shoulder'), attributed to lose of cells viability after the accumulation of oxidative damages during the process, followed by a log-linear decrease (Eq. (10)) [21]. 3) A double log-linear kinetics (Eq. (11)), with a first stage very fast ($k_1 > k_2$) inactivation and a second phase of attenuated inactivation (k_2) [22]. 4) A log-linear region followed by a 'tail' (Eq. (12)) which represents the bacterial population remaining at the end of the experiment [21]. Kinetic parameters obtained for the different equation are comparable, as all results were obtained under the same operational conditions (reactor, illumination, protocols, etc.).

$$Log\left(\frac{N}{N_0}\right) = -k \cdot t \tag{9}$$

$$Log\left(\frac{N}{N_0}\right) = -k_1 \cdot t \left\{ \begin{matrix} 0; N \geq N_0 \\ e^{-k_1(N-SL)}; N < N_0 \end{matrix} \right\} \tag{10}$$

$$Log\left(\frac{N}{N_0}\right) = -k_1 \cdot t; t = (0, t_1); Log\left(\frac{N}{N_0}\right) = -k_2 \cdot t$$

$$\frac{dN}{dt} = -k_1 \cdot N \Rightarrow \frac{N - N_{res}}{N_0 - N_{res}} = e^{k_1 t} \tag{12}$$

Where N/N_0 is the bacteria concentration reductions, k_i is the disinfection kinetic rate and *t* is the time of treatment, N_{res} is the residual population density, and *SL* = Shoulder length (min^{-1}).

3. Results and discusion

Dark experiments were done in IW and MWWE under the same operational conditions of solar experiments as 'control of microorganism's viability'. Results showed no toxicity for any pathogens investigated since microbial viability remained constant for 5 h of treatment time (data not shown). Temperature was monitored in all experiments and it never exceeded 35 °C, therefore thermal inactivation can be discarded. In addition, no regrowth of bacteria was observed when pathogen concentration reached DL.

3.1. Isotonic water (IW)

3.1.1. Solar disinfection, solar/H_2O_2, solar/H_2O_2:EDDS and Fe^{3+}:EDDS

Prior to evaluate the efficiency of solar photo-Fenton with EDDS complex, a series of solar processes were performed in order to analyze the individual effect of all the reagents involved in this process on each bacterium, which is determinant for a further explanation of the inactivation mechanisms. Inactivation of *E. coli* and *E. faecalis* under natural sunlight in IW is shown in Fig. 1a, b, respectively. Experiments in presence of EDDS were carried out at 0.2 mM (24 mg/L of DOC). A similar DOC concentration was added to solar disinfection experiments and solar/H_2O_2 without EDDS using urea, peptone and meet extract as source of organic matter in order to disregard any effect related with the addition of biodegradable organics.

Total inactivation (DL = 4 CFU/mL) by solar disinfection was only obtained in the case of *E. coli* (6 log reduction) with 14.5 kJ/L of Q_{UV}. The concentration of *E. faecalis* only decreased 4 log.

Inactivation kinetics results with H_2O_2/solar (0.3 mM of H_2O_2) and H_2O_2:EDDS/solar (0.3 mM of H_2O_2 and 0.2 mM of EDDS) showed a complete bacterial removal in both cases. pH was ~ 6 and 8 for EDDS absence and presence, respectively. The higher bacterial inactivation rate was obtained in H_2O_2/solar, where both *E. coli* and *E. faecalis* reached DL with 3.1 kJ/L of Q_{UV}. The addition of EDDS showed a significant reduction on both bacterial inactivation, with 4.8 and 10.3 kJ/L of Q_{UV}, respectively to reach DL. H_2O_2 concentration was reduced during the treatments to 0.048 mM and 0.081 mM with and without EDDS, respectively.

The inactivation efficiency of solar/Fe^{3+}:EDDS complex at concentration of 0.1:0.2 mM were also investigated at pH 6 and pH 8. *E. coli* reached DL at both pHs, requiring lower accumulated energy at pH 6 (6.5 kJ/L) compared to pH 8 (10.7 kJ/L). *E. faecalis* inactivation showed the same-trend, achieving a better result at pH 6 (6 log reduction with 13.4 kJ/L of Q_{UV}) than at pH 8 where we only observed a reduction of 2 log with 16.2 kJ/L of Q_{UV}. Iron measured during the solar

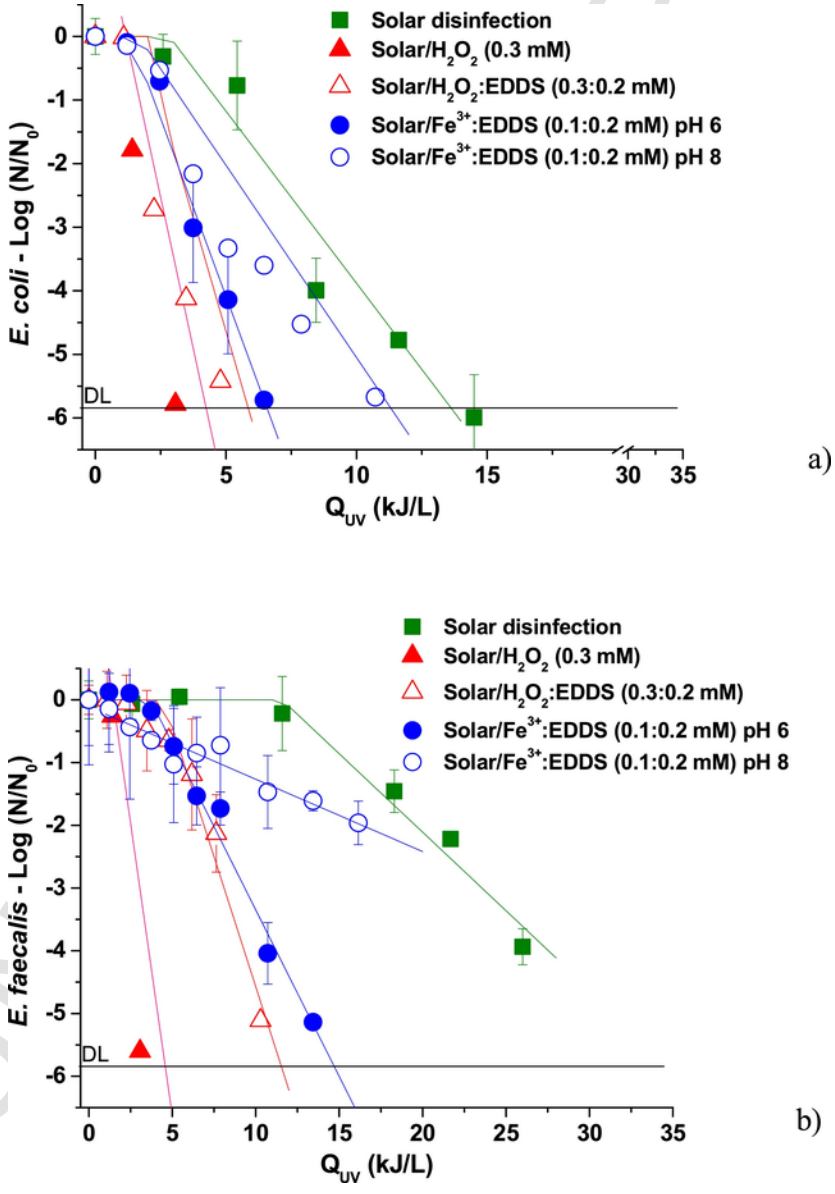


Fig. 1. *E. coli* (a) and *E. faecalis* (b) inactivation kinetics versus Q_{UV} under natural sunlight in IW by several solar processes: solar disinfection, solar/H_2O_2, solar/H_2O_2:EDDS and solar/Fe^{3+}:EDDS.

treatments showed that at time 0 min (T_0), dissolved iron was 0.1 mM. At T_{90}, dissolved Fe at pH 6 remained unchanged (0.1 mM) while at pH 8, Fe in solution decreased 5-fold till 0.02 mM.

3.1.2. Solar photo-Fenton with EDDS complex (Fe^{3+}:EDDS:H_2O_2)

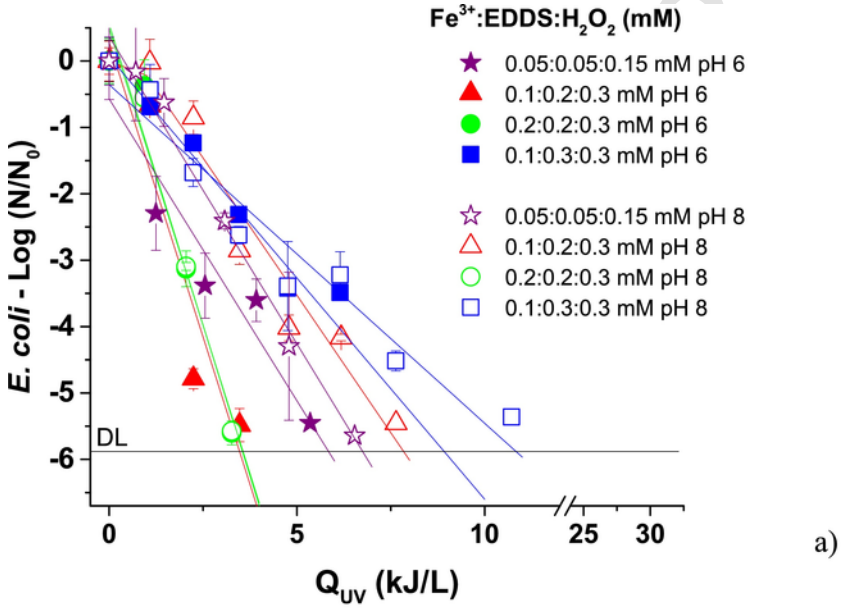
Fig. 2 shows the efficiency of solar photo-Fenton with EDDS to inactivate *E. coli* and *E. faecalis* with several reagent concentrations at pH 6 and pH 8. In general, bacterial inactivation kinetics did not show significant differences between all reagents concentrations tested, being the best results at pH 6. Comparing both bacteria, best inactivation rate was achieved with different reagent´s concentration (Fe^{3+}:EDDS:H_2O_2), i.e., 0.1:0.2:0.3 for *E. coli* and 0.05:0.05:0.15 = 0.1:0.2:0.3 for *E. faecalis*, respectively. Considering the simultaneous bacterial inactivation, we can conclude that 0.1:0.2:0.3 is the best reagent concentration in order to inactivate both pathogens: *E. coli* (Fig. 2a) achieved DL with 3.5 kJ/L of Q_{UV} and *E. faecalis* (Fig. 2b) required 11.9 kJ/L of Q_{UV} to obtain total inactivation at pH 6.

Table 1 shows the iron and H_2O_2 concentration measured during the solar treatments. Dissolved iron was kept longer in solution at pH 6 than at pH 8 in all cases. The best inactivation ratio was found at 0.1:0.2:0.3 of Fe^{3+}:EDDS:H_2O_2, where dissolved Fe was kept for 150 min of treatment.

3.2. Municipal wastewater effluent (MWWE)

3.2.1. Solar disinfection, solar H_2O_2 and H_2O_2:EDDS

Fig. 3 shows pathogens inactivation results by solar disinfection, H_2O_2/solar with H_2O_2 0.15 and 0.3 mM carried out in MWWE with and



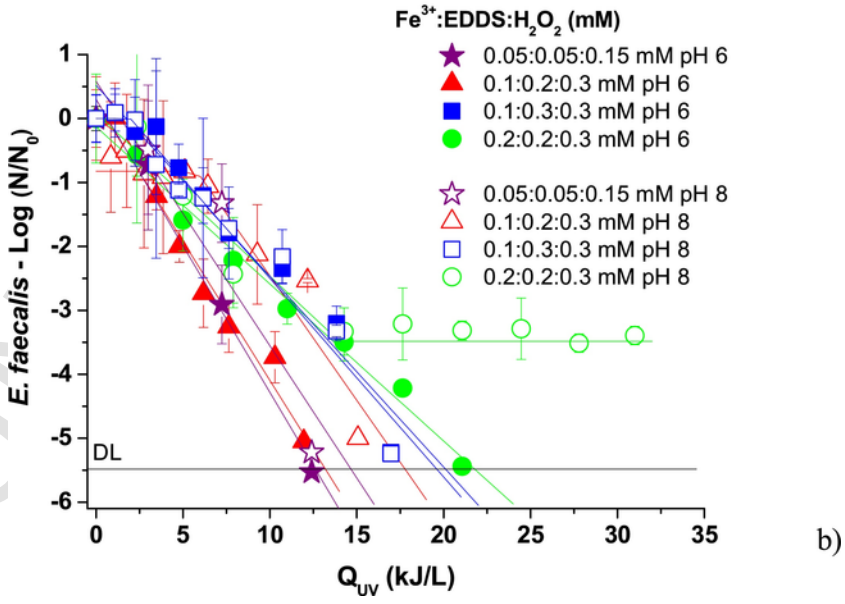


Fig. 2. *E. coli* (a) and *E. faecalis* (b) inactivation kinetics versus Q_{UV} under natural sunlight in IW by photo-Fenton using Fe^{3+}:EDDS:H_2O_2 at several reagent concentrations and pH.

Table 1
Dissolved iron and H_2O_2 (mM) measured during the photo-disinfection experiments in IW and MWWE.

Fe:EDDS:H_2O_2 (mM)	Figure	pH	Dissolved Fe (T_0) (mM)	Time at which Fe is not detectable (min)	Consumed H_2O_2 (mM)
Isotonic water					
0.05:0.05:0.15	2	6.0	0.053	90	0.256
		8.0	0.043	60	0.275
0.1:0.2:0.3	2	6.0	0.100	150	0.425
		8.0	0.100	120	0.398
0.1:0.3:0.3	2	6.0	0.084	120	0.478
		8.0	0.091	100	0.435
0.2:0.2:0.3	2	6.0	0.181	150	0.601
		8.0	0.155	60	0.405
Municipal Wastewater Effluent					
0.05:0.05:0.15	4	7.3	0.044	30	0.290
0.05:0.15*		7.5	0	0	0.150
0.1:0.2:0.3	4	7.6	0.066	30	1.070
0.1:0.3*		7.3	0	0	0.590
0.2:0.2:0.3	4	7.3	0.17	100	0.880
0.2:0.3*		7.5	0	0	0.280

* Corresponding Fe:H_2O_2 concentration, without EDDS.

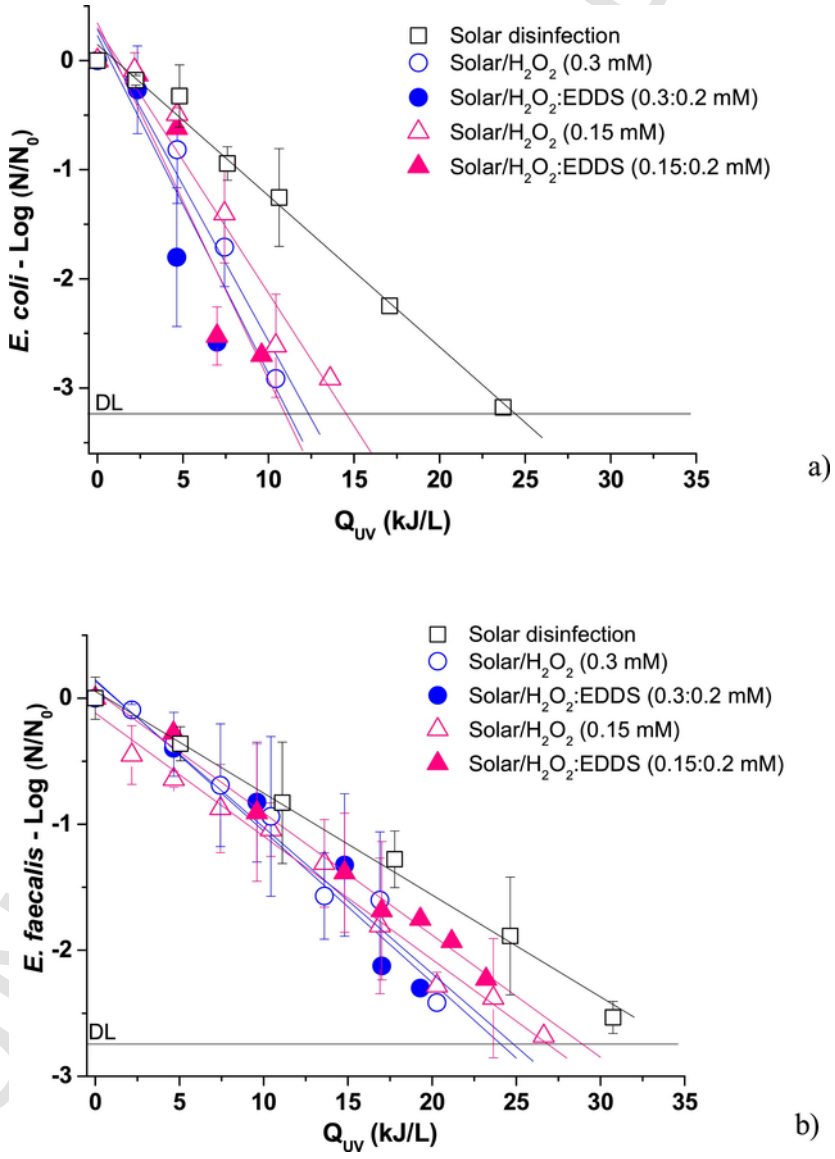


Fig. 3. *E. coli* (a) and *E. faecalis* (b) inactivation kinetics versus Q_{UV} in MWWE under natural solar radiation by several solar processes: solar disinfection, solar/H_2O_2 and solar/H_2O_2:EDDS.

without EDDS. In all cases, the addition of H_2O_2 benefits the treatment compared to solar disinfection. The best inactivation results for *E. coli* (Fig. 3a) were obtained with 0.3 mM of H_2O_2, reaching DL with 10.4 kJ/L of Q_{UV}. The addition of 0.2 mM of EDDS enhanced the inactivation kinetics for both H_2O_2 concentrations: 7.0 kJ/L and 9.6 kJ/L of Q_{UV} were needed to achieve DL at 0.3:0.2 mM and 0.15:0.2 mM of H_2O_2:EDDS, respectively. Complete removal of *E. faecalis* to DL was obtained in all cases, obtaining the best results with 0.3:0.2 mM of H_2O_2:EDDS (19.3 kJ/L of Q_{UV}) (Fig. 3b), followed by 20.3 kJ/L of Q_{UV} for 0.3 mM of H_2O_2. Solar/H_2O_2 at 0.15 mM also reaches DL but with more accumulated energy, 26.6 kJ/L, and 23.2 kJ/L when EDDS was used.

3.2.2. Solar photo-Fenton and solar Fe^{3+}:EDDS:H_2O_2

Inactivation of *E. coli* and *E. faecalis* by photo-Fenton with and without EDDS complex is shown in Fig. 4a and b, respectively. These experiments were done at natural pH (~7.5), and several reagent concentrations were tested. Results showed that the use of EDDS did not enhance the inactivation kinetics as traditional photo-Fenton does. Total removal of *E. coli* was observed in all cases (Fig. 4a). The best concentration tested was 0.1:0.3 mM of Fe:H_2O_2 where DL was reached with 8.2 kJ/L of Q_{UV} whereas the same concentration in the presence of EDDS, required 10.2 kJ/L. For *E. faecalis* inactivation (Fig. 4b), the best inactivation was also found with 0.1:0.3 mM of Fe:H_2O_2 where DL was reached with 23 kJ/L of Q_{UV}. The only case in which DL was reached using EDDS was 0.1:0.2:0.3 mM with 29 kJ/L. Table 1 shows the dissolved iron measured during photo-Fenton treatments with and with-

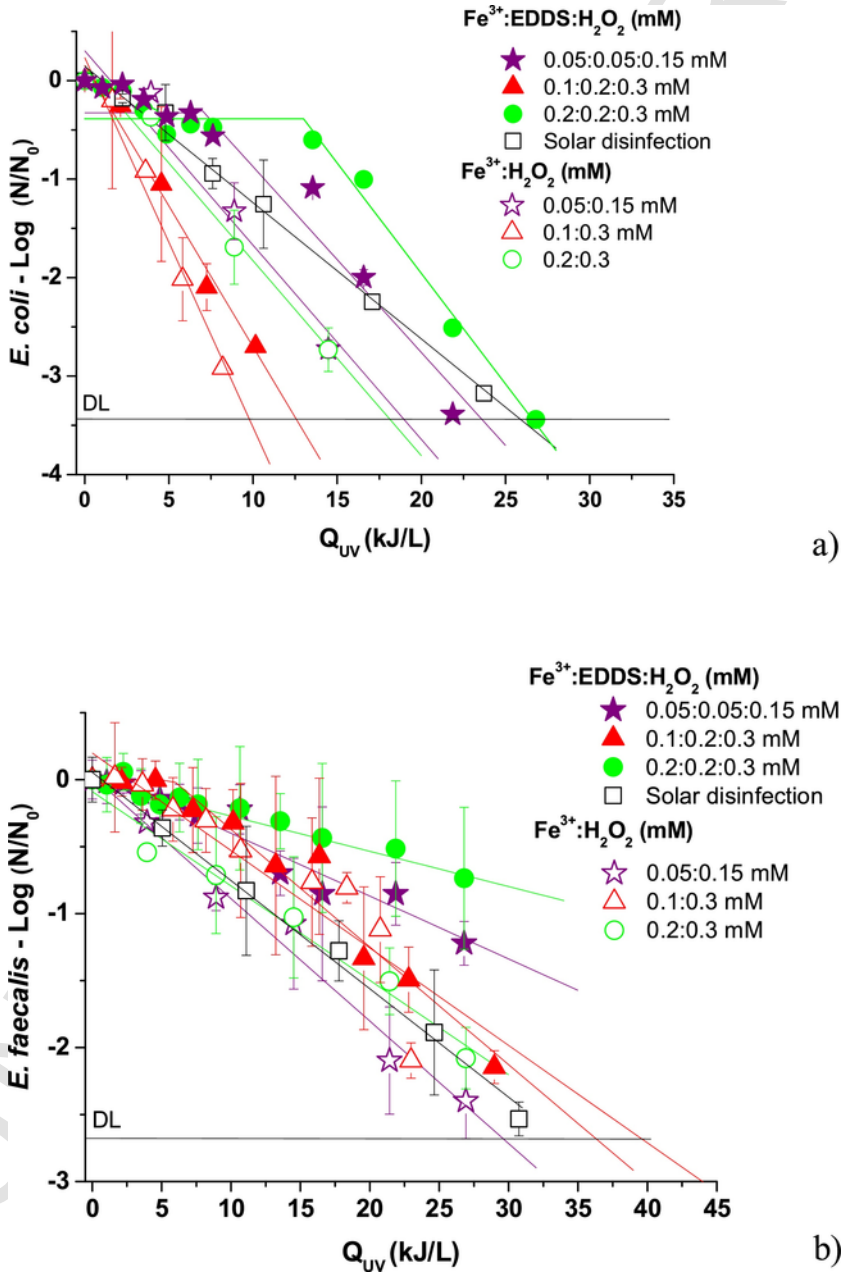


Fig. 4. *E. coli* (a), *E. faecalis* (b) inactivation kinetics versus Q_{UV} under natural sunlight by photo-Fenton with and without EDDS complex in MWWE.

out complex. We can observe that concentration was 0 from the beginning of the experiments in traditional solar photo-Fenton. In the presence of EDDS, dissolved iron was kept in dissolution for at least 30 min of solar treatment.

4. Discussion

The efficiency of photo-Fenton with EDDS at pH 6 and pH 8 was investigated, and results showed that inactivation efficiency was higher at pH 6. Under solar radiation, Fe^{3+}:EDDS is photolysed and Fe^{3+} is transformed to Fe^{2+} (Eq. (3)). This reaction occurs faster at pH 8 than at pH 6 according to higher $\Phi_{HO^\bullet}$. Once Fe^{2+} is formed, it can react with i) EDDS again (Eq. (13)) or ii) H_2O_2 (Eq. (1)). After the decomplexation of Fe:EDDS, the photo-Fenton efficiency will depend on the species of Fe^{3+}/Fe^{2+} formed in the water. It is well known that the most reactive species of iron in water are found at pH 3, whereas at pH 8 almost all iron is precipitated. So, in spite of the higher $\Phi_{HO^\bullet}$ found at pH 8, it seems that the quick loss of iron at this alkaline pH due to Fe^{3+}:EDDS destruction, determines that pathogen inactivation efficiencies by photo-Fenton with EDDS are worst at pH 8 than at pH 6. This effect is observed in Table 1, where Fe decreases significantly more at pH 8 than at pH 6. Most of the decomplexation of Fe-EDDS was produced in less than 90 min. Decomplexation may explain the *E. faecalis* inactivation profile observed in case of 0.2:0.2:0.3 mM at pH 8 in IW, where after 15 kJ/L of Q_{UV} and 60 min. of treatment time, dissolved iron measured was negligible and the bacteria concentration remained stable. In literature, influence of pH on removal of micropollutants by EDDS has reported similar results, determining that pH ranged 5–6 is more beneficial than alkaline water pHs (7–9) [10,13].

Different reagent concentrations were tested in IW in order to find out the best combination of Fe, EDDS and H_2O_2 for water disinfection purpose. Best inactivation was found at concentration ratio of 1:2 of Fe:EDDS, which coincides with previous findings regarding micropollutant removals and coliforms inactivation in MWWE [18,16]. The increase of EDDS concentration in the reagents ratio investigated in IW (0.3 mM) permits keeping iron in solution even at pH 8. However, this concentration did not show a better result than 0.2 mM. This effect may be due to the fact that EDDS could act as competitor for $HO^\bullet$, since $HO^\bullet$ formed during photolysis of the complex could also react with Fe^{3+}:EDDS. This competition is favored in this case, where EDDS concentration is 3-fold higher than iron. This behavior has been described in regards with chemical pollutants degradation by several authors [9,10,13,15,23]. Lowering the EDDS concentration to 0.05 mM (0.05:0.05:0.15 mM Fe:EDDS:H_2O_2) also showed a lower inactivation kinetics than the optimal (0.1:0.2:0.3 mM), demonstrating that the last ratio is the optimum one for the work conditions used to test bacterial inactivation.

Regarding to iron concentration, we must highlight that 0.1 mM was the best option, since when increasing to 0.2 mM or lowering to 0.05 mM, the inactivation efficiency was reduced in both IW and MWWE. This coincides with results reported regarding microcontaminants removal [9], but also, recently, Bianco et al., 2017 reported about *E. faecalis* inactivation with FE(III)EDDS in water. In this work, they concluded that 0.1 mM of Fe:EDDS (ratio 1:1) determined best inactivation kinetic compared to 0.5 and 1 mM [19]. In fact, increasing iron concentration could generate a higher iron precipitation, giving a brown colour to the water and producing suspended particles that may scatter the solar radiation inside the tube, which eventually protected bacteria against solar photons [19,24]. In addition, it has been previously described that for a 6 cm photo-reactor diameter, >0.1 mM of iron could impede radiation by reaching the centre of the photo-reactor, and therefore reducing the efficiency of disinfection.

In general, both the investigated bacteria were completely inactivated (DL reached) by all solar processes in IW. Nevertheless, the inactivation mechanisms may be attributed to the damage accumulation produced by several pathways acting simultaneously; briefly described as follow:

i) Solar disinfection: Photoinactivation of microorganisms under natural sunlight is well known. It is produced by the accumulation of DNA mutation provoked by intracellular $HO^\bullet$ formed due to UVA radiation and the action of other Reactive Oxygen Species (ROS) such as $^1O_2$, O_2^- and H_2O_2 generated and accumulated during solar exposure. In addition, it is also reported inactivation of enzymatic defense like catalase and superoxide dismutase [25–27].

ii) Fe^{3+}:EDDS: when the complex is under sunlight, it is cleaving to generate $EDDS^\bullet$ and Fe^{2+} (Eq. (3)) [12]. In this manner, iron remains dissolved longer than in the absence of a complexing agent. Thus, iron decomplexes gradually, permitting the generation of $HO^\bullet$, responsible of microorganisms' inactivation. After the first photochemical reaction in which EDDS was oxidized and Fe^{3+} was reduced to Fe^{2+}, this Fe^{2+} could oxidize again into Fe^{3+} if EDDS was present in the medium giving Fe^{3+}:EDDS complex again (Eqs. (3)–(5)). Therefore, $HO^\bullet$ radicals for disinfection are produced during this process [9]. Moreover, additional mechanisms can happen when iron is released from the complex, including external $HO^\bullet$ generation by Eqs. (1) and (2) [4]; and internal damages produced by the exciplexes generated in dissolved Fe^{3+} (little amount remaining due to the high pH) on bacterial membrane and the diffusion of Fe^{2+} into the cell, favoring the $HO^\bullet$ generation via intracellular Fenton reaction, and its reaction with intracellular H_2O_2 [28].

iii) H_2O_2 and H_2O_2:EDDS: The bacterial inactivation by addition of H_2O_2 has been widely described in literature [20,29,30]. It could be due to the combined effect of solar disinfection with the increase of intracellular H_2O_2 concentration by free diffusion of H_2O_2 into the cell since it is a stable and uncharged molecule. Once into the cells, H_2O_2 reacts with internal free iron or iron incorporated into enzymes or storage in proteins naturally present in cells generating $HO^\bullet$ by Fenton-Haber-Weiss cycle reactions. Although EDDS can generate $HO^\bullet$ radicals by Eq. (5), this way seems to not be significant in this process as their presence doesn't help the inactivation process. EDDS may react with H_2O_2, scavenging it (Eq. (14)). This reaction may slow down the diffusion of H_2O_2 into the cell, reducing, therefore, the bacterial inactivation kinetics as it was observed in *E. coli* and E. *faecalis* (Fig. 3), compared with the bacterial inactivation by solar/H_2O_2 without EDDS.

iv) Fe:H_2O_2 and Fe:EDDS:H_2O_2: The mechanisms of pathogens inactivation through traditional solar photo-Fenton are produced by the simultaneous action of the $HO^\bullet$ generated by Eqs. (1) and (2) attacking pathogen membranes [31] and the damage generated by ROS formed due to the action of solar UVA and the diffusion of iron and H_2O_2 into the cell (internal photo-Fenton reactions) [32,33]. When EDDS is used for iron complexation, the same Fenton-like reactions explain the $HO^\bullet$ radicals formation, being analogous to those occurred from free Fe^{3+} ion with H_2O_2 (Eqs. (13) and (14)) [14]:

$$Fe^{2+} - EDDS + H_2O_2 \rightarrow Fe^{3+} - EDDS + OH$$

$$Fe^{3+} - EDDS + H_2O_2 \rightarrow Fe^{2+} + EDDS + HO_2$$

In general, *E. faecalis* has shown a higher resistance to be inactivated than *E. coli* in all experimental conditions investigated herein and in both water matrixes evaluated. This behavior has been widely observed in literature [7], and it has been mainly attributed to the different architectures of the cytoplasmic membranes. It is well known that the cell wall of *E. faecalis* (Gram-positive) is thicker than *E. coli* (Gram-negative), which determines, as it has been reported in solar photocatalytic processes, a higher resistance to be inactivated [7,24].

On the other hand, comparing inactivation results in MWWE and IW, lower inactivation kinetics were obtained in MWWE in all treatments, which may be attributed to a reduced catalytic activity by: i) inorganic species like bicarbonates, [34]; ii) water turbidity (average > 11 NTU) screening sunlight, iii) DOC naturally present in MWWE that competes with microbial cells by HO• [33] and iv) additional contribution to DOC by EDDS, which may compete with microbial cells for generated HO• and its reaction with H_2O_2 consuming it (Eq. (14)).

Finally, comparing the inactivation kinetics of both bacteria in all treatments investigated, we observed that the process solar/H_2O_2 with 0.3 mM determined the higher inactivation rate in MWWE (Table 2). Inactivation kinetics with traditional photo-Fenton showed smaller treatment efficiency since Fe dissolved was almost zero even before the solar exposure (Table 1). Therefore, the observed inactivation cannot be considered for a photo-Fenton treatment. In this case, inactivation mechanisms are mainly due to the action of H_2O_2. Even more, in some cases, the inactivation kinetics observed in photo-Fenton were lower compared to H_2O_2/solar treatment which could be due to i) light scattering effect generated by the colour of the water acquired when iron is precipitated (at basic pH) and ii) the screen effect of the particles formed in the water, especially in MWWE which could protect pathogens from solar light action. In literature it is reported that the photolysis of Fe:EDDS is higher in lake water than in IW due to the presence (and reaction) of fulvic and humic acids [35]. Therefore, the photo-degradation of the complex in MWWE could be done at higher rate generating more HO• by Eqs. (3)–(5). In addition, further investigations of the competitive role of organic matter naturally present in MWWE must to be addressed in order to determine its influence on efficiency of EDDS complex for water disinfection.

5. Conclusions

Inactivation results reflect that the use of EDDS improve solar disinfection, even with resistant microorganisms as *E. faecalis*.

For all solar photo-Fenton concentrations, inactivation kinetics were faster at pH 6 than at pH 8, mainly due to iron precipitation, obtaining

Table 2
Microorganism's inactivation rates (k) versus treatment time (minutes) obtained during solar disinfection process in IW and MWWE.

	E. coli					*E. faecalis*				
	k_1 (min^{-1})	R_1^2	SL (min.)	DL	Model #	k_1 (min^{-1})	R_1^2	SL (min.)	DL	Model #
TREATMENT	**ISOTONIC WATER**									
SODIS	0.054±0.013	0.951	60	Y	2	0.020±0.002	0.989	120	N	2
Complex pH6	0.098±0.008	0.989	15	Y	2	0.049±0.004	0.990	45	Y	2
Complex pH8	0.055±0.005	0.982	15	Y	2	0.010±0.001	0.968	–	N	1
0.3 H_2O_2	0.096±0.021	0.976		Y	1	**0.093±0.049**	**0.886**		**Y**	**1**
0.3H_2O_2/0.2EDDS	0.117±0.016	0.982	15	Y	2	0.076±0.011	0.981	60	Y	2
0.05:0.05:0.15 pH6	0.081±0.013	0.965	–	Y	1	0.031±0.005	0.974	–	Y	1
0.05:0.05:0.15 pH8	0.059±0.005	0.983	–	Y	1	0.028±0.010	0.898	–	Y	1
0.1:0.2:0.3 pH6	**0.137±0.032**	**0.949**	–	**Y**	**1**	0.036±0.003	0.988	15	Y	2
0.1:0.2:0.3 pH8	0.073±0.008	0.978	15	Y	2	0.041±0.010	0.947	90	Y	2
0.1:0.3:0.3 pH6	0.056±0.004	0.984	–	Y	1	0.028±0.003	0.966	–	Y	1
0.1:0.3:0.3 pH8	0.046±0.004	0.982	–	Y	1	0.031±0.003	0.973	30	Y	2
0.2:0.2:0.3 pH6	0.130±0.025	0.964	–	Y	1	0.025±0.001	0.996	–	Y	1
0.2:0.2:0.3 pH8	0.128±0.023	0.971	–	Y	1	0.025±0.003	0.975	–	N	4
		MWWE								
SODIS	0.012±0.001	0.987	–	Y	1	0.008±0.000	0.995	–	N	1
0.3 H_2O_2	**0.032±0.007**	**0.951**	–	**Y**	**1**	**0.011±0.001**	**0.977**	–	**Y**	**1**
0.3 H_2O_2/0.2EDDS	0.031±0.005	0.970	–	Y	1	0.010±0.001	0.976	–	Y	1
0.15 H_2O_2	0.021±0.003	0.959	–	Y	1	0.010±0.000	0.992	–	Y	1
0.15 H_2O_2/0.2EDDS	0.026±0.006	0.937	–	Y	1	0.008±0.000	0.994	–	Y	1
0.05:0.05:0.15	0.018±0.002	0.976	75	Y	2	0.004±0.000	0.969	–	Y	1
0.1:0.2:0.3	0.024±0.002	0.985	–	Y	1	0.010±0.001	0.958	30	N	2
0.2:0.2:0.3	0.006±0.001	0.918	–	Y	3	0.002±0.000	0.978	–	N	1
0.05:0.15	0.016±0.004	0.953	–	Y	1	0.008±0.001	0.982	–	Y	1
0.1:0.3	0.026±0.003	0.977	–	Y	1	0.006±0.001	0.909	–	Y	1
0.2:0.3	0.016±0.002	0.980	–	Y	1	0.006±0.001	0.986	–	N	1

Model: 1 (Log lin); 2 (Shoulder-Log); 3 (Log lin-Log lin); 4 (Log-Tail).

best inactivation kinetic at concentration of 01:0.2:0.3 mM of Fe^{3+}:EDDS:H_2O_2.

It has been proved that H_2O_2/solar is a good and efficient alternative to disinfect water, especially in MWWE.

In MWWE, photo-Fenton disinfection's efficiency was not enhanced by the presence of EDDS even though iron keeps in solution longer. Effect of different organic and inorganic components of MWWE should be investigated in detail in order to understand better the effect each of them has and clarify which are detrimental and which beneficial for the process.

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